genic bacteria, actively secreted and/or released by lysis of microbial cells found in the small and/or large intestine;

[0014] (vi) increased stability when expressed in a heterologous host such as a yeast such as a yeast belonging to the genera *Aspergillus, Saccharomyces, Kluyveromyces, Hansenula* or *Pichia* (by virtue of increased resistance to yeast proteases);

[0015] (vii) reduced risk of adverse immune reactions;

[0016] (viii) reduced production costs;

[0017] (ix) improved treatment and/or prevention of intestinal infection or autoimmune and/or inflammatory diseases;

[0018] (x) improved patient acceptance and long term compliance;

[0019] (xi) improved yield during recombinant production;

[0020] (xii) improved bioactivity and/or biodistribution;

[0021] (xiii) reduced required dosage;

[0022] (xiv) suitability for, and improved properties for, use in a pharmaceutical;

[0023] (xv) suitability for, and improved properties for, use in a functional food.

SUMMARY OF THE INVENTION

[0024] The present inventors have produced surprisingly advantageous polypeptides comprising immunoglobulin chain variable domains, suitable for oral administration. These polypeptides are particularly advantageous due to their increased intestinal stability (i.e. increased stability in the intestinal tract). It may be expected that these polypeptides have particular utility in the prevention or treatment of diseases of the gastrointestinal tract such as autoimmune and/or inflammatory disease such as inflammatory bowel disease, or in the prevention or treatment of infection from intestinal tract resident pathogenic microbe. Also provided are methods of increasing the intestinal stability of a polypeptide comprising an immunoglobulin chain variable domain and methods of making a polypeptide comprising an immunoglobulin chain variable domain having increased stability.

[0025] Accordingly, the present invention provides a polypeptide comprising an immunoglobulin chain variable domain comprising three complementarity determining regions (CDR1-CDR3) and four framework regions, wherein: (a) at least one lysine residue in CDR1, CDR2 and/or CDR3 has been substituted with at least one histidine residue, and/or (b) at least one arginine residue in CDR1, CDR2 and/or CDR3 has been substituted with at least one histidine residue; wherein the polypeptide has increased intestinal stability relative to a corresponding polypeptide not having said histidine substitutions.

[0026] Also provided is a method of increasing the intestinal stability of a polypeptide comprising an immunoglobulin chain variable domain, wherein the immunoglobulin chain variable domain comprises three complementarity determining regions (CDR1-CDR3) and four framework regions, wherein the method comprises the step of substituting: (a) at least one lysine residue in CDR1, CDR2 and/or CDR3 with at least one histidine residue, and/or (b) at least one arginine residue in CDR1, CDR2 and/or CDR3 with at least one histidine residue.

[0027] Also provided is a method of making a polypeptide comprising an immunoglobulin chain variable domain, wherein the immunoglobulin chain variable domain comprises three complementarity determining regions (CDR1-CDR3) and four framework regions, wherein the method comprises the step of substituting: (a) at least one lysine residue in CDR1, CDR2 and/or CDR3 with at least one histidine residue, and/or (b) at least one arginine residue in CDR1, CDR2 and/or CDR3 with at least one histidine residue wherein the polypeptide has increased intestinal stability relative to a corresponding polypeptide not having said histidine substitutions.

[0028] Also provided is a polypeptide comprising a region which is capable of binding a target with high affinity wherein: (a) at least one lysine residue in the region has been substituted with at least one histidine residue, and/or (b) at least one arginine residue in the region has been substituted with at least one histidine residue; wherein the polypeptide has increased intestinal stability relative to a corresponding polypeptide not having said histidine substitutions.

DESCRIPTION OF THE FIGURES

[0029] FIG. **1**—Example TcdA dose-response curve on Vero cells

[0030] FIG. **2**A—Potency of anti-TNF ICVDs Q65B1, ID8F-EV, ID43F and ID44F (Experiment 1) against human TNF in the TNFR2/TNF interference ELISA

[0031] FIG. **2**B—Potency of anti-TNF ICVDs Q65B1 and ID8F-EV (Experiment 2) against human TNF in the TNFR2/TNF interference ELISA

[0032] FIG. **3**A—Stability of anti-TNF ICVDs Q65B1, ID8F-EV, ID43F and ID44F in mouse small intestinal supernatant after 6 hours incubation

[0033] FIG. **3**B—Stability of anti-TNF ICVDs Q65B1 and ID8F-EV in human faecal and mouse small intestinal supernatant after 16 hour incubation

[0034] FIG. **4**—Potency of ICVDs ID32F and ID34F against human TNF in the TNFR2/TNF interference ELISA

[0035] FIG. **5**A—Stability of anti-TNF ICVDs ID32F and ID34F in mouse small intestinal supernatant after 16 hours incubation

[0036] FIG. **5**B—Stability of anti-TNF ICVDs ID32F and ID34F in human faecal supernatant pool 4 after 16 hours incubation

[0037] FIG. **6**A—TcdB 027 neutralisation by ID45B-ID50B in the Vero cell cytotoxicity assay

[0038] FIG. **6**B—Stability of anti-TcdB ICVDs ID45B-ID50B in human faecal supernatant pool 4 after 30 minutes incubation, analysed by western blot

[0039] FIG. **7**—TcdB 027 neutralisation by ID2B, ID20B, ID21B and ID22B in the Vero cell cytotoxicity assay

[0040] FIG. **8**A—ID2B trypsin assay—stained polyacrylamide gel

[0041] FIG. **8**B—ID20B and ID21B trypsin assays—stained polyacrylamide gels

[0042] FIG. **8**C—ID22B trypsin assay—stained polyacrylamide gel

[0043] FIG. **9**—Stability of anti-TcdB ICVDs ID2B and ID21B in human faecal supernatants after 1 hour incubation

[0044] FIG. **10**A—TcdB 027 neutralisation by ID1B, ID24B, ID25B and ID27B in the Vero cell cytotoxicity assay

[0045] FIG. **10**B—Stability of anti-TcdB ICVDs ID1B, ID24B, ID25B and ID27B in human faecal supernatant pool 2 after 1 hour incubation